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(71) Applicant (for all designated States except US):
PHARMEXA A/S [DK/DK]; Kogle Allé 6, DK-2970
Hørsholm (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BRATT, Tomas
[SE/DK]; c/o Pharmexa A/S, Kogle Allé 6, DK-2970 Hørsholm (DK). KLYSNER, Steen [DK/DK]; c/o Pharmexa A/S, Kogle Allé 6, DK-2970 Hørsholm (DK). NIELSEN, Finn [DK/DK]; c/o Pharmexa A/S, Kogle Allé 6, DK-2970 Hørsholm (DK). MOURITSEN, Søren [DK/DK]; Høveltej 78, DK-3460 Birkerød (DK). VOLDBORG, Bjørn [DK/DK]; c/o Pharmexa A/S, Kogle Allé 6, DK-2970 Hørsholm (DK).

(74) Agent: INSPICOS A/S; Bøge Allé 5, P.O. Box 45, DK-2970 Hørsholm (DK).

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(54) Title: IMMUNOGENIC HUMAN TNF ALPHA ANALOGUES WITH REDUCED CYTOTOXICITY AND METHODS OF THEIR PREPARATION

(57) Abstract: The present invention provides for an immunogenic analogue of a human TNF α protein, wherein said analogue comprises an immunogenized monomeric TNF α polypeptide or TNF α di- or trimer, and wherein the analogue further comprises a toxicity reducing or abolishing mutation selected from the group consisting of Y87S, D143N or A145R, the amino acid numbering setting out from the N-terminal valine in human TNF α . The invention also provides for a nucleic acid fragment encoding the analogue as well as to vectors and transformed cells useful in the preparation of the analogue. Also disclosed are methods of down-regulating TNF α in a subject in need thereof.

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A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07K14/525 C12N15/28 A61K38/19 A61K48/00 A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, EMBASE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DALUM I ET AL: "THERAPEUTIC ANTIBODIES ELICITED BY IMMUNIZATION AGAINST TNF-ALPHA" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 17, no. 7, 1999, pages 666-669, XP000910155 ISSN: 1087-0156 page 666 page 668, right-hand column, last paragraph – page 669, right-hand column, paragraph 1</p> <p style="text-align: center;">-/-</p>	1-33

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

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Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Strobel, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LOETSCHER HANSRUEDI ET AL: "Human tumor necrosis factor alpha (TNF-alpha) mutants with exclusive specificity for the 55-kDa or 75-kDa TNF receptors" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 35, 1993, pages 26350-26357, XP002304715 ISSN: 0021-9258 abstract page 26355, right-hand column, paragraph 2 page 26356, right-hand column, paragraph 3; figure 4 -----	1-33
Y	ZHANG X M ET AL: "SITE-DIRECTED MUTATIONAL ANALYSIS OF HUMAN TUMOR NECROSIS FACTOR-ALPHA RECEPTOR BINDING SITE AND STRUCTURE-FUNCTIONAL RELATIONSHIP" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 267, no. 33, 25 November 1992 (1992-11-25), pages 24069-24075, XP002922635 ISSN: 0021-9258 abstract page 24074, right-hand column, paragraph 1; table 1 -----	1-33
A	DATABASE EMBL 'Online! EBI; 13 December 1990 (1990-12-13), XP002304718 retrieved from EBI Database accession no. AAR06359 abstract	1-9
A	& PATENT ABSTRACTS OF JAPAN vol. 014, no. 446 (C-0763), 25 September 1990 (1990-09-25) & JP 02 177896 A (TEIJIN LTD), 10 July 1990 (1990-07-10) abstract	1-9
A	-& DATABASE WPI Derwent Publications Ltd., London, GB; AN 1990-252060 XP002304720 & JP 02 177896 A (TEIJIN) 10 July 1990 (1990-07-10) abstract -----	1-9
P,X	DATABASE EMBL 'Online! EBI; 22 September 2003 (2003-09-22), XP002304719 retrieved from EBI Database accession no. AA030467 abstract	1-9

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	& WO 03/042244 A (BRATT TOMAS ; KLYSNER STEEN (DK); PHARMEXA AS (DK); VOLDBORG BJOERN (D) 22 May 2003 (2003-05-22) page 34, line 23 – page 47, line 5; claims 21,22 ----- P,X NIELSEN FINN S ET AL: "A therapeutic HTN α vaccine induces anti-TN α antibodies that inhibit signalling through the TN α : TNF receptor pathway." EUROPEAN CYTOKINE NETWORK, vol. 14, no. Supplement 3, September 2003 (2003-09), page 16, XP009039612 & ANNUAL MEETING OF THE INTERNATIONAL CYTOKINE SOCIETY; DUBLIN, IRELAND; SEPTEMBER 20-24, 2003 ISSN: 1148-5493 the whole document ----- T NIELSEN FINN STAUSHOLM ET AL: "Insertion of foreign T cell epitopes in human tumor necrosis factor alpha with minimal effect on protein structure and biological activity" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 279, no. 32, 6 August 2004 (2004-08-06), pages 33593-33600, XP002304716 ISSN: 0021-9258 the whole document -----	1-33 1-33 1-33

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the International search was carried out on the basis of:
 - a. type of material
 - a sequence listing
 - table(s) related to the sequence listing
 - b. format of material
 - in written format
 - in computer readable form
 - c. time of filing/furnishing
 - contained in the international application as filed
 - filed together with the international application in computer readable form
 - furnished subsequently to this Authority for the purpose of search
2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 1 (partially), 10-24 (all partially)
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 10-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. Claims Nos.: 1 (partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 10-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

Claims Nos.: 1 (partially)

Present claim 1, alternative (h), relates to a product defined by reference to a desirable characteristic or property, namely introduction of a foreign MHC class II binding amino acid sequence so as to stabilise the monomer structure by increasing the hydrophobicity of the trimeric interaction interface of the claimed TNF alpha analogue.

The claim covers all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the TNF alpha analogues carrying a foreign MHC class II binding amino acid sequence in general.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK2004/000329

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
JP 02177896	A	10-07-1990	NONE	
WO 03042244	A	22-05-2003	CA 2467052 A1 WO 03042244 A2 EP 1448598 A2 US 2003185845 A1	22-05-2003 22-05-2003 25-08-2004 02-10-2003